Association of Vitamin B$_{12}$ and Metformin in Type II Diabetes Patients in Bahrain

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Abstract

**Objective:** Metformin therapy is the gold standard treatment for type 2 diabetic patients. Long term use of metformin has been associated with vitamin B$_{12}$ deficiency. We aimed to study the level of vitamin B$_{12}$ in type 2 diabetic patients on metformin, and to determine other factors affecting the level of vitamin B$_{12}$.

**Methodology:** Out of 344 patients with type 2 diabetes that were interviewed from January to December 2015; 304 (91.3%) were investigated for vitamin B$_{12}$ levels deficiency. The patient’s lab results from 2015-2016 were reviewed from the electronic hospital information system, patient data file.

**Result:** Most of the patients, 56.4% had a normal vitamin B$_{12}$ level. In 91% of the patients the treating physician had already investigated for serum vitamin B$_{12}$ level. Dyslipidemia and hypertension were significantly more (p=0.009 and 0.022 respectively) in patients with normal vitamin B$_{12}$ level. Percentage of normal B$_{12}$ level patients was higher (p=0.001) even in more than 20 years of metformin use; 46.7% of more than 20 years Metformin users had vitamin B$_{12}$ level within 301-450 pg/mL and 33.3% had more than 450 pg/mL.

**Conclusion:** We recommend routine screening for basal vitamin B$_{12}$ level for all patients with type 2 diabetes mellitus (T2DM) at the initiation of metformin therapy with subsequent annual follow up of the level.

Keywords: Vitamin B$_{12}$; Metformin; Type II diabetes; Bahrain

Introduction

Metformin is considered as the first-line antidiabetic agent in the treatment of type 2 diabetes mellitus (T2DM) due to its effect on glucose and lipid metabolism [1] simultaneously with the major protective role it plays against life threatening complications. It acts as an insulin sensitizer on insulin-targeted tissues; liver, muscle, and adipose tissues and reduces insulin resistance. Additionally it offers protection from cardiovascular diseases and heart failure [2,3]. Given its safety and efficacy record, it is routinely prescribed to approximately 120 million type 2 diabetes patients world-wide [4].

However various reports have recognized long-term treatment with metformin as a pharmacological cause of vitamin B$_{12}$ deficiency [5-7]. On the other hand, the exact prevalence of vitamin B$_{12}$ deficiency among diabetic patients described in different literature is not constant.

Diabetic neuropathy, affecting more than 90% of the patients is the commonest complication of diabetes [6]. The fact that vitamin B$_{12}$ deficient neuropathy and diabetic neuropathy cannot be distinguished clinically [7] renders it difficult to determine which of the two pathology is actually responsible for the neuropathy in a diabetic patient with a coexisting vitamin B$_{12}$ deficiency. Long term treatment with metformin therefore increases the chance of vitamin B$_{12}$ neuropathy be misdiagnosed as diabetic neuropathy or it may contribute in worsening the diabetic neuropathy [7].

In addition to neuropathy long term metformin therapy has been linked also to the development of anaemia [8]. Once again the mechanism of this could be the metformin induced vitamin B$_{12}$ deficiency as it is a well-recognized cause of megaloblastic anemia.

As vitamin B$_{12}$ deficient neuropathy and diabetic neuropathy both present with similar indistinguishable clinical manifestations, the objectives of this study were: 1) to determine the level of Vitamin B$_{12}$ in T2DM patients on metformin; 2) to identify additional factors that may affect the Vitamin B$_{12}$ level in T2DM patients on metformin; 3) the effect of metformin use in the level of vitamin B$_{12}$ in type 2 diabetic patients.

Material and Methods

Type 2 diabetic adult patients attending the diabetic clinic of primary care department of a Bahrain Defense Force Royal Medical Services Military Hospital were randomly recruited between January 2015 to December 2015. Each participant was interviewed with a structured questionnaire and was asked about: personal data, duration of T2DM, duration and dose of metformin, other risk factors (dyslipidemia, hypertension, cerebrovascular diseases, Coronary Vascular Disease, smoking, hypothyrodsim, use of insulin and nephropathy) and symptoms of peripheral neuropathy. The interview consisted of a verbal consent followed by direct questions by the treating physician who recorded the answers manually. To evaluate
cerebrovascular diseases, patients were questioned about past history of cerebrovascular accident, transient ischemic attack or stroke. Peripheral neuropathy was assessed by asking the patient about presence of tingling sensation, heat sensation or paresthesia of the lower limb.

A total of 344 patients who were above the age of 30 years, who are being treated by metformin were interviewed. Serum Vitamin B\textsubscript{12} Assay was ordered by the treating physician to 305 patients, so the rest of them were excluded. Type 1 diabetic patients were not included in this study. Nor were the T2DM below 30 years of age, not receiving metformin, or suffering from chronic renal insufficiency defined by serum creatinine level less than 3. Patients with a prior history of gastrostomy, bariatric surgery, ileum resection or Crohn’s disease were also excluded.

After the completion of interview lab results from 2015-2016 were reviewed from the electronic hospital information system patient data file. Level of vitamin B\textsubscript{12}, HbA1C, hemoglobin were recorded. Patients were considered low Vitamin B\textsubscript{12} level if it were less than 300 pg/ml. Ethics Board approval was obtained from Bahrain Defence Force Royal Medical Services prior to commencement of this cross-sectional study.

Statistical Analysis

Data were collected and stored in a spreadsheet using Microsoft Excel 2010® software. Data management and coding were then done in Excel. Data were analyzed using SPSS® version 20.0 (IBM Inc., Chicago, Illinois, USA).

Descriptive analysis was done, where categorical variables were presented in the form of frequencies and percentages. Chi\textsuperscript{2} test was used to test for differences between the groups and test for associations. Results were compared between the Vitamin B\textsubscript{12} deficient group and Normal Vitamin B\textsubscript{12} group in two ways. 1) Number and percentage of each variable was calculated in each group and compared within the same group and 2) Percentage of each variable was compared between the 2 two groups. A p-value below 0.05 was interpreted as an indicator of statistical significance.

Result

The demographic data of the 305 participants’ are presented in Table 1. The highest number of participants, (46.1%) was from 50 to 60 years old. Most of the patients, 172 (56.4%) had a normal vitamin B\textsubscript{12} level (p=0.023). The majority of patients, 193 (57.8%) were male. Bahraini nationals represented 68.9% of participants. Greater percentage (59.3%) of the patients were obese with a BMI higher than 30. About 32% of patients had T2DM duration of 1-5 years and 6-10yrs. Similarly longest period of time treated with metformin is 1-5 years 36.2% and 6-10yrs 33.2%. Majority of the patients, 220 (65.9%) were receiving total daily dose above 2000 mg. A good number of patients, 166 (49.7%) reported symptoms of peripheral neuropathy. In spite of laboratory result revealing Hb level below 12 mg/dl in 18% of the participants, only 12% of the patient presented with clinically apparent anaemia. The level of last HbA1c was between 6 to 7% in 105 (31.4%) participants.

<table>
<thead>
<tr>
<th>Characteristic Total (n)</th>
<th>Vitamin B\textsubscript{12} deficiency</th>
<th>Normal Vitamin B\textsubscript{12}</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interviewed 334</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B\textsubscript{12} Assay 305</td>
<td>133 (43.6)</td>
<td>172 (56.4)</td>
<td>0.028*</td>
</tr>
<tr>
<td>Sex (male, %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>81 (60.9)</td>
<td>93 (54.1)</td>
<td>0.232</td>
</tr>
<tr>
<td>Female</td>
<td>52 (39.1)</td>
<td>79 (45.9)</td>
<td></td>
</tr>
<tr>
<td>Age (years%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-60</td>
<td>96 (44)</td>
<td>122 (55)</td>
<td>0.034*</td>
</tr>
<tr>
<td>Above 60</td>
<td>37 (42.5)</td>
<td>50 (57.5)</td>
<td>0.049*</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.5-24.9</td>
<td>11 (37.9)</td>
<td>18 (62.1)</td>
<td>0.068</td>
</tr>
<tr>
<td>25-29.9</td>
<td>36 (39.6)</td>
<td>55 (60.4)</td>
<td>0.005*</td>
</tr>
<tr>
<td>&gt;30</td>
<td>85 (46.7)</td>
<td>97 (53.3)</td>
<td>0.209</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Below 1 year</td>
<td>3 (50.0)</td>
<td>3 (50.0)</td>
<td>0.998</td>
</tr>
<tr>
<td>1-5 years</td>
<td>43 (43.0)</td>
<td>57 (57.0)</td>
<td>0.048*</td>
</tr>
<tr>
<td>6-10 years</td>
<td>44 (47.8)</td>
<td>48 (52.2)</td>
<td>0.552</td>
</tr>
<tr>
<td>11-20 years</td>
<td>33 (41.3)</td>
<td>47 (58.8)</td>
<td>0.027*</td>
</tr>
<tr>
<td>Duration of Metformin use</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Below 1 year 5 (45.5) 6 (54.5) 0.68
1-5 years 45 (40.2) 67 (59.8) 0.003*
6-10 years 46 (47.4) 51 (52.6) 0.47
11-20 years 34 (47.4) 36 (52.6) 0.741
Above 20 years 3 (20.0) 12 (80.0) 0.001*

Table 1: General characteristics of patients with and without vitamin B₁₂ deficiency.

Risk Factors | Deficient n(%) | Normal n(%) | P value
--- | --- | --- | ---
Dyslipidemia | 120 (90.2) | 150 (87.2) | 0.412
Hypertension | 90 (67.7) | 113 (65.7) | 0.717
Insulin treatment | 24 (18.0) | 27 (15.7) | 0.586
Smoking | 19 (14.3) | 24 (14.0) | 0.934
CVD | 15 (11.3) | 17 (9.9) | 0.694
Hypothyroidism | 7 (5.3) | 12 (7.0) | 0.539
Cerebrovascular d | 8 (6.0) | 6 (3.5) | 0.296
Periph. Neurop. Neuropathy | 9 (6.8) | 5 (2.9) | 0.11
Nephropathy | 120 (90.2) | 150 (87.2) | 0.412

Table 2: Number and Percentage of patients in Normal and Deficient group with different risk factors.

Risk Factors | Deficient n(%) | Normal n(%) | P value
--- | --- | --- | ---
Dyslipidemia | 120 (44.4) | 150 (55.6) | 0.009*
Hypertension | 90 (44.3) | 113 (55.7) | 0.022*
Insulin treatment | 24 (47.1) | 27 (52.9) | 0.56
Smoking | 19 (44.2) | 24 (55.8) | 0.285
CVD | 15 (46.9) | 17 (53.1) | 0.623
Hypothyroidism | 7 (36.8) | 12 (63.2) | 0.108
Cerebrovascular d | 8 (57.1) | 6 (42.9) | 0.461
Periph. Neuropathy | 65 (43.9) | 83 (56.1) | 0.036*
Nephropathy | 9 (64.3) | 5 (35.7) | 0.137

Table 3: Number and Percentage of Risk factors in patients with and without vitamin B₁₂ deficiency.

Low vitamin B₁₂ was observed in 133 participants which represents 43.6% of the total number. In comparing the patients with low vitamin B₁₂ and the normal, normal vitamin B₁₂ was significantly more frequent in age 41 and above (p=0.044), females 79 (60.3%) (p<0.001), non-Bahraini population 58 (49.8%) (p=0.007). Fifty five (60.4%) patients with a BMI between 25-29.9 had normal vitamin B₁₂ level. None of the risk factors we investigated-dyslipidemia, hypertension, insulin treatment, smoking, CVD, hypothyroidism, nephropathy showed any statistically significant association with vitamin B₁₂ deficiency (Table 2). On the other hand dyslipidemia, hypertension was found significantly more with a p-value of 0.009 and 0.022 respectively, in patients with normal vitamin B₁₂ level. Only 17 patients representing 5.1% of the sample complained of cerebrovascular diseases, and 8(57.1%) of them had low vitamin B₁₂ compared to 6 (42.9%) normal, but the difference was not statistically significant (Table 3). The correlation between serum B₁₂ levels and the duration of metformin was determined in two different ways. Firstly we compared the patients who were using metformin for the same duration. There was significant difference in the 1 to 5 years users and above 20 years users. At the same time in both this group the percentage of participant with normal B₁₂ level were significantly higher. Most of the patients, 59.8% (p=0.003) using metformin for the last 1-5 years had normal B₁₂ level. Similarly, 15 patients were receiving metformin for more than 20 years and 12 among them (80%) showed normal vitamin B₁₂ level (p=0.001) (Figure 1). However when the duration was evaluated against the different levels of metformin, all of the patients who had a level below 100 were using metformin from 11 to 20 years, which was only 2.3% of that subgroup. On the other hand the longest time of metformin users which in this study is more than 20 years; none of them had serum B₁₂ level below 100. Moreover 46.7% of this group had vitamin B₁₂ level within 301-450 pg/mL and 33.3% had more than 450 pg/mL (Figure 2). Also the patients receiving metformin less than one year; showed 45.5% participant B₁₂ level within 301-450 pg/mL (Figure 2).
Figure 1: Percentage of Vitamin B12 Deficient and Normal patients according to duration of metformin use. Significant difference in 1-5 years (p=0.003) and above 20 years (p=0.001).

Figure 2: Percentage of Patients on different Vitamin B12 level (pg/mL) according to the duration of Metformin Use.

Figure 3: Percentage of Patients on different Vitamin B12 level according to the Dose of Metformin.

No statistically significant difference existed between the HbA1c level of the low vitamin B12 and normal group, larger portion of both groups had HbA1c level between 6-7%; 37.6% for low and 29.1% for the normal vitamin B12. Then again, when compared separately for each HbA1c level, significantly larger portion (64.6%) of the normal vitamin B12 level patients had HbA1c level higher than 8% (p=0.004) in Table 4.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Vitamin B12 deficiency, n (%) ≤ 300 pg/mL</th>
<th>B12, n (%) ≤ 600 pg/mL</th>
<th>Normal Vitamin B12, n (%) &gt;300 pg/mL</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level of last HbA1c</td>
<td>Comparison of deficient and normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Below 5.9%</td>
<td>6 (4.5)</td>
<td>9 (5.2)</td>
<td></td>
<td>0.445</td>
</tr>
<tr>
<td>6-7%</td>
<td>50 (37.6)</td>
<td>50 (29.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.1-8%</td>
<td>36 (27.1)</td>
<td>44 (25.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.1-9%</td>
<td>17 (12.8)</td>
<td>31 (18.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Above 9%</td>
<td>24 (18.0)</td>
<td>38 (22.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level of last HbA1c</td>
<td>Comparison between HbA1c Levels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Below 5.9%</td>
<td>6 (40.0)</td>
<td>9 (60.0)</td>
<td></td>
<td>0.282</td>
</tr>
<tr>
<td>6-7%</td>
<td>50 (50.0)</td>
<td>50 (50.0)</td>
<td></td>
<td>0.998</td>
</tr>
<tr>
<td>7.1-8%</td>
<td>36 (45.0)</td>
<td>44 (55.0)</td>
<td></td>
<td>0.207</td>
</tr>
<tr>
<td>8.1-9%</td>
<td>17 (35.4)</td>
<td>31 (64.6)</td>
<td></td>
<td>0.004*</td>
</tr>
<tr>
<td>Above 9%</td>
<td>24 (38.7)</td>
<td>38 (61.3)</td>
<td></td>
<td>0.012*</td>
</tr>
</tbody>
</table>

Table 4: Correlation between level of HbA1c and level of Vitamin B12.
Anemia and hemoglobin level did not have any statistically significant difference between low and normal vitamin B₁₂.

**Discussion**

In our study we found that low vitamin B₁₂ level was prevalent in 43.6% of T2DM patients on metformin. Higher current daily dose and duration of metformin use have positive association with low vitamin B₁₂ level. The present study could not establish any association between the low vitamin B₁₂ level and BMI, Symptoms of peripheral neuropathy, anemia and hemoglobin level. The normal vitamin B₁₂ level patients had higher HbA1c level. Also this study found that more than 90% of patients were already checked for vitamin B₁₂ deficiency.

Prevalence of vitamin B₁₂ deficiency among diabetic patients shows a wide range of variation from 5.8% to 52% [5,8-15]. This discrepancy between different studies may be due to the fact that, each of the studies followed their own reference ranges for normal value of vitamin B₁₂, along with difference in age, study settings, and dose and duration of use of metformin [7].

De Jager et al. [5,8,9] studied patients from Netherlands, United States and Sweden. Their prevalence was much lower than the present study; 5.8 to 9.9%. Another study recent study conducted in South Africa found a moderately higher prevalence of 28.1% [7]. The difference might not reflect the difference in geographical distribution. Rather this might be explained by their cut off point ranging between 145-150 pmol/L compare to our 300 pmol/L. We chose this cutoff point following a recent study that conducted in a large sample of 700 patients in Korea, chose serum level ≤ 300 pmol/L their cut off point for vitamin B₁₂ deficiency [19]. Even with this high cutoff point their prevalence was 9.5%. The high prevalence in the current study might be explained by both the early detection of vitamin B₁₂ deficiency and high number of deficient patients. More than ninety one percent of the participants were already investigated for vitamin B₁₂ deficiency by their treating physicians.

In this study we did not find statistically significant difference in presence of cerebrovascular diseases between the participants with normal and low vitamin B₁₂ levels. When compared with total study population, in both deficient and normal vitamin D group, 48% was positive for peripheral neuropathy (p=0.987). In view of the fact that peripheral neuropathy is by itself is a complication of the T2DM, we further analyzed the data of the patients showing symptoms of peripheral neuropathy independently. That analysis revealed that significantly larger portion (56.1%) of the patients with peripheral neuropathy infact had normal vitamin B₁₂ level (p=0.036). Similar to our study the result of another study published in 2016 could not establish any association with the presence of diabetic peripheral neuropathy in T2DM patients and use of metformin [16]. Similar observation was reported from a study comparing metformin user and nonuser diabetic patients [17]. There was no difference in neurological function between the both groups.

The present study found that larger daily dose of metformin has a positive correlation with vitamin B₁₂ deficiency (Figure 3) [18]. Several previous studies have already reported this inverse effect of dose of metformin on serum vitamin B₁₂ level [7,12,15,19-21].

In our study we found weak association of duration of metformin use with low vitamin B₁₂ level. All the patients who had below 100 vitamin B₁₂ level were receiving metformin for 11-20 years. On the other hand almost half of the patients who were receiving metformin for more than 20 years had a serum vitamin B₁₂ level between 300 to 450 pg/mL (Figure 2). Several other studies have recognized longer duration of metformin use as an independent risk factor for vitamin B₁₂ deficiency in T2DM patients [5,7,19-22]. de Jager et al. [5] revealed that a-19% (95% confidence interval -24% to -14%; P<0.001) mean decrease of vitamin B₁₂ level was observed in long term metformin users in comparison to placebo group. Ahmed et al. [7] demonstrated that metformin use duration was significantly longer (11 vs. 8 years, P=0.015) in B₁₂-deficient participants. Ko et al. [19] observed that metformin use of more than 4 years is associated with Vitamin B₁₂ deficiency (P<0.001). Similar to their observation, Ting et al. [21] demonstrated that Vitamin B₁₂ deficiency has significant association with metformin use of 3 years or more the adjusted odds ratio was (2.39, 95% confidence interval, 1.46-3.91) (P=0.001). Contrary to all these observations, Chen et al. [17] did not find any significant association between metformin duration and vitamin B₁₂ deficiency. In our study when we compared vitamin B₁₂ deficient and normal patients receiving metformin for less than 1 year, 1-5 years, 6-10 years, 11-20 years and longer than 20 years. Our result revealed no significant association between these two groups in each year category (Figure 1).

Level of HbA1c in relation to serum vitamin B₁₂ levels was also calculated. We demonstrated that high HbA1c level is found more frequently in T2DM patients with a normal vitamin B₁₂ level. Previously two other studies [7,14] supported similar association. Ahmed et al. [7] tried to explain this association from the perspective of patient compliance. The gastrointestinal adverse effects of metformin may promote poor compliance in patients, especially with high dose of the drug. The poor or noncompliance may consequently lead to poor glycaemic control and higher HbA1c.

One major limitation of this study is that we did not look for the number of patients already on vitamin B₁₂ supplementation (some of the patient may take vitamin B₁₂ supplementation from other hospitals) there is a possibility that a good number of patients are receiving it. And that might be the reason for our study result not showing much significant difference between the variables of both the groups.

**Conclusion**

Our result indicates that vitamin B₁₂ deficiency is prevalent among type 2 diabetic patients treated with metformin, especially those on high dose of medication and longer duration. There is no association of vitamin B₁₂ deficiency with diabetic neuropathy. Also this study revealed that the majority of the patients are being investigated for Vitamin B₁₂ deficiency, which is a very positive sign for the health care system. So we recommend routine screening for basal vitamin B₁₂ level for all patients with T2DM at the initiation of metformin therapy with subsequent annual follow up of the level. Although we could not establish any association between diabetic neuropathy and Vitamin B₁₂ deficiency, further studies are needed to identify symptoms that will help to distinguish these two separate entities.

**References**

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